

STUDIES ON THE EMBRYOLOGY AND
RELATIONSHIPS OF SOUTH AFRICAN GENERA OF
THE HAEMODORACEAE: *DILATRIS* Berg. AND
WACHENDORFIA Burm.

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ABSTRACT.

The literature on the embryology of genera placed in the Haemodoraceae by various taxonomists is reviewed. In a number of cases a knowledge of the embryological characteristics has contributed to a better understanding of the relationships of these genera.

Embryological investigations in *Dilatrix pillansii* Barker have shown that a periplasmodium is formed in the anthers and the pollen-grains develop successively; the ovule is sessile, orthotropous, bitegmic and crassinucellate; a parietal cell and a row of four megaspores are formed, the embryo-sac development is of the normal type, and the endosperm development helobial. A small chalazal haustorium develops from the primary basal endosperm cell.

The information of Dellert on the embryological development of *Wachendorfia paniculata* Burm. is supplemented.

The embryological investigations indicate that (1) *Dilatrix* and *Wachendorfia* are very close in their relationships, (2) these two genera are also closely related to *Xiphidium* and *Anigosanthus*, and (3) that these genera stand closer to the tribe Hypoxideae than to any other group. Embryological results therefore support the placement of *Dilatrix* and *Wachendorfia* together in one tribe and also of the four genera in one family as Benthams and Hooker and Hutchinson have done.

It has been repeatedly suggested in the past that the Haemodoraceae is not a natural family and probably not of monophyletic origin. Baillon (1891), for example, states that it is a heterogeneous family which probably ought to disappear for being composed of fragments from divers groups. Engler (1897) calls it an unnatural family, the genera of which should be moved partly to the Liliaceae and partly to the Amaryllidaceae. Lotsy (1911) agrees with Pax (1889) and thinks it to be "wenig einheitlich", and most probably of polyphyletic origin; "ja, sie ist möglicher-

weise mit der Zeit ganz aufzulösen und unter die Liliaceae und Amaryllidaceae zu verteilen". Pax (1930) considers it either an old family of which a few remaining groups have been left, or "eine nicht einheitliche gestaltete Familie polyphyletischen Ursprungs".

On the other hand, Hutchinson (1944) considers it a natural family as he has delimited it. He states, "... I am firmly of the opinion that the two tribes" (i.e. Haemodoreae and Conostyleae which Pax separated into different families) "should be again associated to form the Haemodoraceae. Thus constituted, but with the genus *Aletris* removed to the Liliaceae, the family is natural and homogeneous, not only in its facies, but in its general distribution which is predominantly austral, ..."

The delimitation of the family by various systematists has been very different. See the summary in Table I.

REVIEW OF THE EMBRYOLOGICAL LITERATURE.

In an attempt to discover the natural affinities of the Haemodoraceae genera, the embryological development of some has already been worked out. As could be expected, this information has led to some valuable suggestions about the relationships of these genera.

Stenar (1927, 1938) has shown on embryological evidence that a close relationship exists between *Xiphidium* and *Anigosanthus*, and Dellert (1933) has shown that *Wachendorfia* stands close to *Anigosanthus*. Both Dellert and Stenar further find enough evidence to suggest that these respective genera are also related to the tribe Hypoxideae of the Amaryllidaceae, and that they can be classed together.

Pauridia has been found on embryological (de Vos 1948, 1949) and other evidence (cf. Schulze, 1893; Brackett, 1923) to be closely allied to some South African members of the Hypoxideae. This genus should be placed in this group—as has been done by Bentham and Hooker, Hutchinson and by Baker in Thiselton-Dyer's "Flora Capensis".

Cave (1952) has demonstrated that the early stages of the embryology of *Odontostomum* agree with that of *Cyanastrum* (Fries, 1919; Nietsch, 1941), which in Engler and Prantl's system is the only genus of the family Cyanastraceae, and which Hutchinson on the other hand places in the Tecophilaeaceae together with *Odontostomum* and the four other genera of the tribe Conanthereae. According to Cave these results substantiate Hutchinson's placement of *Odontostomum* and *Cyanastrum* together in one family. The seed anatomy of *Cyanastrum* is unique in the Liliiflorae, as it has no endosperm, the embryo is very large, and the cells in the chalaza proliferate after fertilization to form a large tissue containing food reserves (Fries, Nietsch). It is unfortunate that stages after fertilization in *Odontostomum* were not available for investigation.

TABLE I. THE DELIMITATION OF THE HAEMODORACEAE BY VARIOUS TAXONOMISTS.

	Endlicher 1836-40, 1842.	Bentham & Hooker, 1883.	Engler & Prantl, 1930.	Hutchin- son, 1944.	Baker in Flora Capensis, 1896.	In Adam- son & Salter, 1950.	Phillips 1951.	Distribu- tion.
Haemodorum ..	Hae	Hae-Euhae	Hae	Hae-Haem				Austr.
Wachendorfia ..	"	"	"	"	Hae-Euhae	Hae	Hae	S. Afr.
Schiekia ..	"	"	"	"				Amer.
Hagenbachia ..	"	"	"	"				Amer.
Dilatis ..	"	"	"	"	"	"	"	S. Afr.
Lachnanthes ..	"	"	"	"				Amer.
Barberetta ..	"	"	"	"	"		"	S. Afr.
Xiphidium ..	"	"	"	"				Amer.
Lanaria ..	"	"	Ama-Cono	"	"		Ama	S. Afr.
Phlebocarya ..	"	"	"	"				Austr.
Pauridia ..	? Hypox	? Ama-Hyp	Hae	Hypox	Ama-Hyp	Ama	Hae	S. Afr.
Tribonanthes ..	Hae	Hae-Cono	Ama-Cono	Hae-Cono				Austr.
Conostylis ..	"	"	"	"				"
Blancoa ..	"	"	"	"				"
Anigosanthus ..	"	"	"	"				"
Macropidia ..	"	"	"	"				"
Lophiola ..	"	"	"	"				Amer.
Aletris ..	"	"	Lil-Ale	Lil-Nar				Amer., Asia
Peliosanthes ..	Smi	Hae-Oph	Lil-Mon	Lil-Pel				Asia
Ophiopogon ..	"	"	"	Lil-Oph				"
Liriope ..	"	"	"	"				"
Sansevieria ..	Lil	"	Lil-Dra	Agav	Hae-Oph		Lil	Afr., Asia
Conanthera ..	"	Hae-Con	Ama-Con	Teco				Amer.
Cyanella ..	"	"	"	"	Hae-Con	Hae	Ama	S. Afr.
Zephyra ..	"	"	"	"				Amer.
Tecophilaea ..	Ir. aff.	"	"	"				"
Odontostomum	"	"	Lil-Asph	"				"

KEY TO ABBREVIATIONS.—*Families*: Agav(aceae), Ama(ryllidaceae), Hae(modoraceae), Ir(ideae), Hypox(idaceae), Lil(iaceae), Smi(laceae), Teco(philaeaceae). *Subfamilies*: Ale(troideae), Mon(doideae). *Tribes*: Asph(odeleae), Con(anthereae), Cono(stylideae) or Cono(styleae), Dra(caeneae), Euhae(modoreae) Haem(odoreae), Hyp(oxideae), Nar(theciae), Oph(iopogoneae), Pel(iosantheae).

Cyanella, the only other genus of Hutchinson's Tecophilaeaceae which has been studied embryologically (de Vos, 1950), shows a somewhat similar early development: no periplasmodium develops in the anthers and the walls in the p.m. cells form simultaneously¹; the ovule shows a parietal cell, normal type of embryo-sac development, and nuclear endosperm development. It differs in the possession of many ovules in each chamber many of which ripen, and from *Cyanastrum* in the possession of a well developed endosperm. More embryological investigations in this group are needed.

¹ de Vos, unpublished.

Sansevieria, a genus which Bentham and Hooker placed in the Haemodoraceae, shows in its embryological development its close relationship with *Dracaena* (Stenar, 1942; Wunderlich, 1950) and not with the Ophiopogoneae as Bentham and Hooker thought. This supports Engler and Prantl and Hutchinson who group these two genera together.

Alettris, now usually considered to be a genus of the Liliaceae, has been studied by Ono (1928, 1929, cited by Schnarf, 1931). According to Schnarf's review, *Alettris* has been found to possess a parietal cell, normal type of embryo-sac development, and helobial endosperm. Whether Ono was able to make any suggestions about the relationship of this genus is not known, as his papers have not been available.

These are the only Haemodoraceae genera which have been examined embryologically up to now, as far as could be found out. From this short review it is clear that the Haemodoraceae as constituted by most systematists is probably not a homogeneous group. More embryological investigations, the term used in its broadest sense as defined by Cave (1953), to include both sporophytic and gametophytic structures that are concerned in sexual reproduction, are highly desirable in other genera of the Haemodoraceae, especially in the Australian and New World groups. Such information will probably help greatly in discovering the natural affinities of the genera.

In the present paper the development of the ovule, seed and pollen in *Dilatris* is described. As Dellert's description of the embryology of *Wachendorfia* is not complete because of the scarcity of material she had available, and as I had already started investigating *Wachendorfia* before Dellert's paper came to my notice, a short revision of the embryology of this genus is also given. This leaves only *Lanaria* and *Barberetta* of the South African genera still to be studied.

INVESTIGATION.

Method.—Young flower buds and older isolated ovaries were fixed in FAA. In still older stages ovules were dissected out before fixation to get rid of the hairy ovary walls. After embedding in paraffin wax sections were cut 10—15 μ in thickness, and stained either with Heidenhain's iron-alum haematoxylin or with Delafield's haematoxylin.

DILATRIS PILLANSII BARKER.

Pollen.—Before reduction division in the pollen-mother-cells the tapetal cells become binucleate and start migrating into the pollen-sacs to form a periplasmodium. This reaches its maximum development when the young pollen-grains are separating after the reduction division

and forming an exine. Remains of the periplasmodium are still to be seen some time after the exine has developed.

The cell-wall formation in the p.m. cells is successive, the first wall being formed after the first reduction division.

The generative cell which is very small and more or less pear-shaped, is formed in one corner of the pollen-grain against the end of the distal wall (Fig. 25). The vegetative nucleus, which at this stage lies near the generative cell, is much larger and often shows two nucleoli. The nuclei of the two cells stain to about the same intensity.

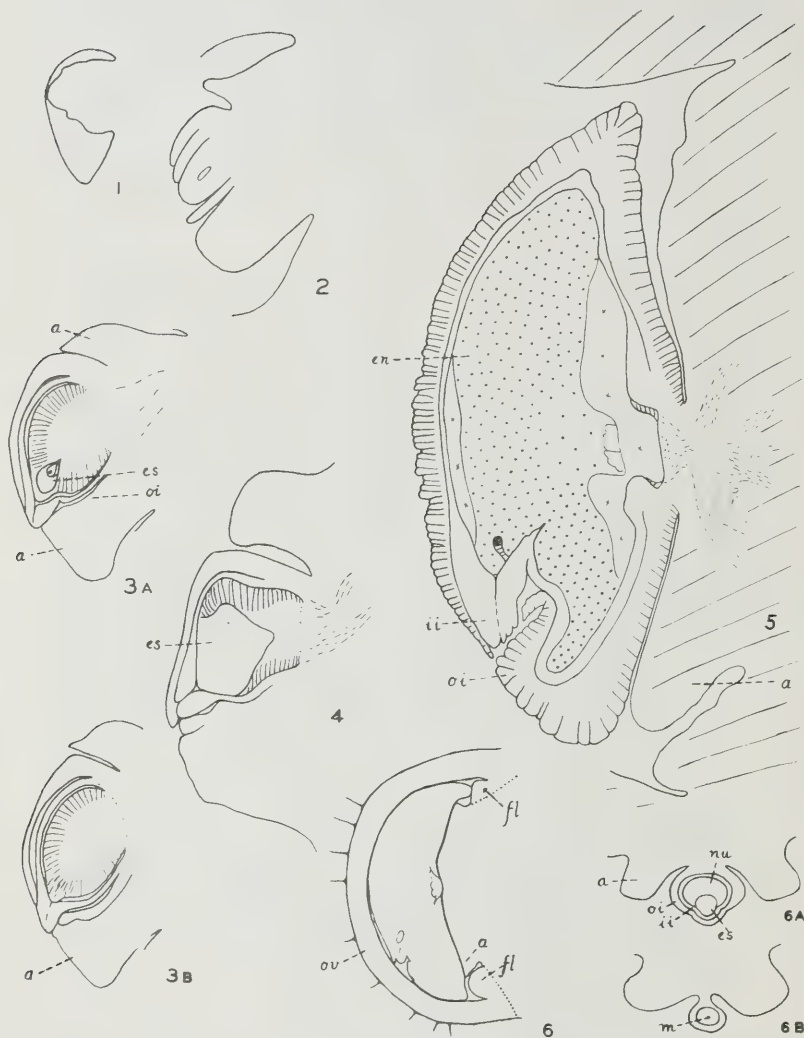
Position and form of ovule.—The ovary is inferior and three-chambered and has a single ovule on a large axile placenta situated in the upper part of each chamber. The ovule is sessile and is attached by means of its broad chalaza to the placenta. It is orthotropous but slightly bent and pendulous, the micropyle pointing downwards (Figs. 2–4). A sharp dent occurs in the integuments on one side near the micropyle.

A broad vascular bundle containing a large number of scalariform tracheids runs from the placenta into the chalaza. Here it expands and lies in contact with the entire broad base of the nucellus (Fig. 4). It does not branch off into the integuments.

The placenta enlarges rapidly and forms a thick cushion-like outgrowth round the base of the ovule, and at the time of fertilization the micropyle nearly touches it (Figs. 3A, B). It can be compared with the aril found in various plant groups, but it does not enlarge to form a third integument. After fertilization the ovule overtakes it in growth.

FIGS. 1–6. *Dilatris pillansii*, sections through developing ovule and seed. FIG. 1, very early stage with nucellus and primordia of integuments, $\times 80$; 2, slightly older with megaspore-mother-cell, developing integuments, and placental outgrowth connected below with the outer integument, in median longitudinal section, $\times 80$; 3A–B, mature embryo-sac stage; 3A, median longitudinal section with the lower part of the outer integument and placental outgrowth connected; 3B, near median section, two sections removed from 3A, the outer integument free from the placental outgrowth, $\times 50$; 4, ovule after fertilization showing enlarging embryo-sac and disappearing nucellus, near median, $\times 50$; 5, near median section of developing seed with endosperm tissue, small embryo, and degenerating chalazal haustorium, $\times 35$; x denoting spaces not filled up with tissue; 6, longitudinal section of fertile ovary chamber showing nearly ripe seed and part of flange; dotted lines indicate where the carpel walls break at seed dispersal, $\times 8$; 6A & 6B, cross-section through ovule and placental outgrowth to show connection: 6A, through lower part of ovule, 6B, through micropylar region, $\times 11$; a, placental outgrowth (aril); en, endosperm; es, embryo-sac; fl, flange; *i*, inner integument; m, micropyle; nu, nucellus; oi, outer integument; ov, ovary wall.

Integuments.—The two integuments develop from the base of the young nucellus, the inner one beginning its development slightly before the outer. Both consist of two cell layers, except at the base of the outer integument and the tip of the inner, where they are thicker. The micropyle is usually formed by the inner integument only, as the outer



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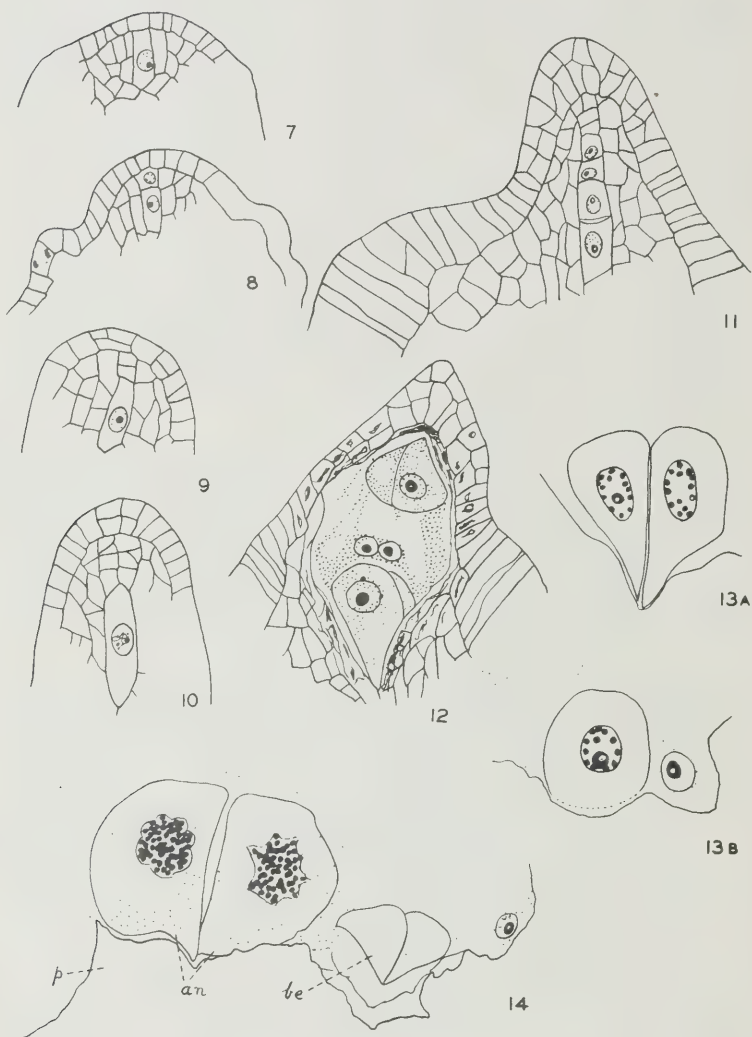
is slightly shorter than the inner on the side away from the placenta (Figs. 3A, B). In a few cases the outer integument overlaps the inner and forms a very short continuation of the micropylar canal (Fig. 4).

Where the pendulous ovule touches the placenta, the outer integument is attached to the placental outgrowth by means of a narrow strip of parenchymatous tissue in the median longitudinal line (Figs. 2—4, 6A, B). This begins to develop in an early stage and is still to be seen in the nearly ripe seed. (The seed shown in Fig. 5 still has it, two sections removed from the section figured—on account of its narrowness a section not quite median does not show the connection.) This connection perhaps indicates that the orthotropous condition of the ovule is not primary, having been developed from an anatropous condition by reduction. In a median longitudinal section it looks somewhat like the raphe of an anatropous ovule, except that no vascular bundle runs through it. No reference in the literature could be found to any such state in other orthotropous ovules.

Nucellus and embryo-sac.—The nucellus begins its development as a hemispherical projection from the placenta. A subepidermal archesporial cell is differentiated which divides periclinally to form a megaspore-mother-cell and a parietal cell. A small cell-complex consisting of several layers originates from the latter by periclinal and antichlinal divisions. The epidermal cells at the tip of the nucellus divide once periclinally. Ultimately the megaspore-mother-cell therefore lies embedded under five or six layers of nucellar tissue (Figs. 7—10).

FIGS. 7—14. *Dilatris pillansii*, development of female gametophyte. FIG. 7, subepidermal archesporial cell; 8, megaspore-mother-cell and parietal cell; 9, slightly older megaspore-mother-cell and developing nucellar cap formed by divisions in the parietal cell and nucellar epidermis; 10, still older stage with megaspore-mother-cell in Prophase I; 11, four megaspores formed after reduction division; note the palisade-shaped nucellar epidermal cells lower down; 12, mature embryo-sac, the egg-cell not shown; nucellar cells beginning to disintegrate; 13A & B, base of embryo-sac in two adjacent sections showing enlarging antipodal cells, with primary endosperm nucleus in 13B; 14, base of embryo-sac with two basal endosperm cells (developing chalazal haustorium), two hypertrophied antipodal cells on a small postament, and one free nucleus of the upper endosperm chamber; all $\times 350$; *an*, antipodal cells; *be*, basal endosperm cells (chalazal haustorium); *p*, postament.

The megaspore-mother-cell elongates and undergoes reduction division. Four megaspores are formed in a row, and the chalazal one develops into a normal 8-nucleate embryo-sac (Figs. 11—13) which at first has a narrow pointed base. The three large antipodal cells are inversely pear-shaped and lie in one level with their pointed bases directed towards the chalaza. The two polar nuclei lie just above them and sometimes in contact with them. At the micropylar end the egg-apparatus shows no extraordinary features. Before fertilization the embryo-sac does not



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enlarge much and takes up only a small space in the upper part of the nucellus.

Fertilization was not seen, but remains of what probably was the pollen-tube were found in the micropyle of one ovule.

The nucellus is large and crassinucellate. At first it is ovoid but later becomes somewhat pear-shaped with a massive base and a pointed tip. The widening of the base is brought about by an elongation of the epidermal cells in the anticlinal direction in the lower half of the nucellus (Figs. 3A, 4, 12). These cells elongate up to six or seven times their width, thus becoming palisade-shaped.

In early stages the base of the nucellus consists of small meristematic cells rich in protoplasmic contents. It is sharply defined from the chalaza, as the vascular elements in the latter end abruptly against the meristematic base of the nucellus.

The developing embryo-sac absorbs the nucellar cells along its sides as far as the nucellar epidermis. After fertilization this process is accelerated and somewhat later only the nucellar cap at the micropyle consisting of the nucellar epidermis, and the palisade-shaped epidermal cells near the base are left. Still later they too are absorbed and no nucellar tissue remains in the ripening seed.

The antipodal cells increase in size at about the time of fertilization and then disappear gradually. Before their disappearance the adjacent nucellar cells are absorbed by the embryo-sac, leaving the antipodals on a small pedestal of nucellar cells (the "postament", Schnarf, 1929, p. 59) (Fig. 14). On account of the faster absorption of nucellar cells towards the chalaza a small chalazal endosperm haustorium is formed, and the postament with the antipodal cells is left on the farther side where they degenerate slowly. At the same time the longitudinal axis of the embryo-sac changes so that the haustorium which at first is somewhat lateral, becomes basal.

Endosperm.—The two polar nuclei fuse at about the time of fertilization and after fertilization the primary endosperm nucleus moves from its position near the antipodal cells to that side of the embryo-sac where the small haustorium is being formed towards the placenta (Fig. 13B).

The endosperm formation is helobial. Although the first division of the primary endosperm nucleus was not observed, a small basal endosperm cell is formed after this mitosis. Two, and shortly afterwards four very large basal cells are formed from the first one by vertical divisions (Fig. 14, 15). They contain much protoplasm and each has at least two large hypertrophied nuclei. They form the small chalazal haustorium which projects towards the placenta. After the basal nucellar tissue has been absorbed they nearly touch the chalazal vascular bundle and

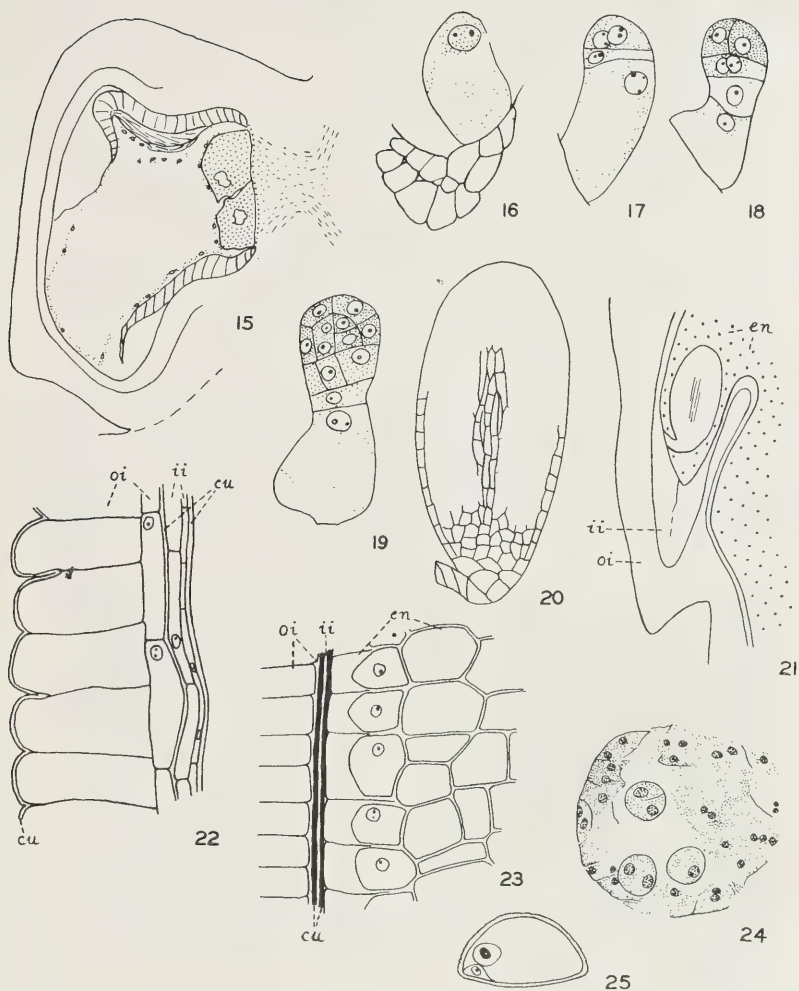
probably help to convey food materials to the developing endosperm. Later, when the seed has reached its full size but is not yet quite ripe, these four cells are absorbed.

In the large upper cell formed after the first division of the primary endosperm nucleus, repeated nuclear divisions take place and a large number of nuclei are formed in the thin layer of cytoplasm which lies against the wall of the embryo-sac (Fig. 15). In one case one of these nuclei and some cytoplasm was seen to intrude into one of the degenerating antipodal cells. Cell-wall formation is progressive from the base towards the micropyle. Later the embryo-sac becomes filled with endosperm tissue by repeated nuclear and cell-divisions.

FIGS. 15—25, *Dilatris pillansii*, development of embryo and seed, and gametogenesis in anther. FIG. 15, submedian longitudinal section of ovule after fertilization showing two of the four cells of the chalazal haustorium and numerous endosperm nuclei, $\times 350$; 16, zygote attached to nucellar cap, $\times 350$; 17, embryo in quadrant stage, $\times 350$; 18, embryo in octant stage, with only two of the four terminal cells shown, $\times 350$; 19, embryo in a later stage where the derivatives of the first apical and first basal cell can still be identified, $\times 350$; 20, longitudinal section of still undifferentiated embryo of nearly ripe seed, with short, bent suspensor and elongated central cells, $\times 250$; 21, embryo, same as in Fig. 20, showing its position in a small sac or diverticulum, with surrounding endosperm and integuments, $\times 50$; 22, section through seed-coat of nearly ripe seed, $\times 250$; 23, section through outer layers of endosperm and adjacent seed-coat, $\times 250$; 24, transverse section through pollen-sac showing periplasmodium and pollen-mother-cells in interphase of reduction division, $\times 350$; 25, young pollen-grain with generative and vegetative cell, $\times 400$; *cu*, cuticular layers; *en*, endosperm; *ii*, inner integument; *oi*, outer integument.

Embryo.—After the formation of cell-walls in the endosperm the fertilized egg-cell begins to divide. The first division is transverse and this is followed by a vertical division in the apical cell and a transverse one in the basal cell (Fig. 17). The octant stage is reached after another vertical division in both apical cells at right angles to the first, a vertical division in the middle cell, and a transverse one in the basal cell (Fig. 18, where two of the four terminal cells are shown). The cell-divisions after this stage were not followed. The derivatives of the apical cell of the two-celled proembryo are richer in protoplasmic contents than those of the basal cell; they divide repeatedly and form the larger part of the embryo. The cells derived from the middle cell of the four-celled proembryo also take part in the embryo formation, which therefore conforms to the Asterad or to the Crucifer (Onagrad) type. The suspensor which is derived from the basal cell of the four-celled proembryo, remains short and consists of three or four cells.

In the ripe seed the embryo is oval in longitudinal section and undifferentiated except for a slight elongation of the central cells. It lies in a small sac or diverticulum formed by the micropylar tip of the embryo-sac which is folded flat against the enlarging endosperm. It is covered by a thin layer of endosperm on the outside and the suspensor is bent (Figs. 20, 21).



Seed.—Usually only one seed matures in an ovary, the other two ovules aborting. Degeneration of these ovules usually takes place after the embryo-sac has developed but may sometimes occur earlier. One ovule, for example, was found with four disintegrating megaspores. When degeneration occurs in the mature embryo-sac, the egg-apparatus usually disintegrates while the antipodal cells become hypertrophied and may increase up to $70\ \mu$ in length, with nuclei $35\ \mu$ in diameter.

After fertilization the shape of the viable ovule changes completely from a more or less spherical to a round inverted watchglass-shaped structure with a concave lower (chalazal) and a convex upper surface. This is caused by an enormous growth in width so that its transverse diameter is later about four times its height. It fills the entire ovary chamber completely, and the micropylar tip becomes flattened against its convex upper surface, thus forming the small sac in which the embryo is situated (Figs. 5, 6, 21). The large placenta pushes against the concave lower surface. The chalaza and hilum are in the centre of this concave surface, and the axis of the seed from chalaza to micropyle is oblique.

Barker (1940) has observed that the ovule "... is clasped by a flange which projects inwards round the entire edge of each carpel. The carpel-wall containing the ripe seed thus held in place by the flange begins to separate from the receptacle, starting at the base and continuing upwards, until it finally breaks away completely with the perianth attached to the apex, thus forming a mechanism suitable for dispersal by wind."

The present developmental studies show that the flange is formed by an enlargement and lignification of a zone of cells of the septa separating the fertile ovary chamber from the others, and of the axis above and below the placenta (Fig. 6). This flange then holds the seed in place when the fertile carpel with its seed breaks away from the receptacle by a tear which develops in the thin part of the septum next to the flange (the tear is indicated by dotted lines in Fig. 6). The placenta is left behind attached to the receptacle.

The seed-coat is formed mainly by the outer epidermis of the outer integument (Fig. 22). These cells become palisade-shaped and contain a brown substance. The inner layer of cells of this integument and both layers of the inner integument become crushed and disorganized (Fig. 23). Three layers of cuticle are present: one on the outside, the second between two of the crushed layers (i.e. between the two integuments), and the third just inside the seed-coat.

The endosperm in the ripe seed is copious and contains starch, protein and oil. The cell-walls are thin, except for the outer layer of endosperm cells (Fig. 23) which have very thick outer walls which give a positive reaction with Schultze's solution. These cells contain small, round

globoid-like bodies which give neither a starch, protein, nor oil reaction. It is uncertain what their nature is.

Germination.—Seeds collected between January and March 1949 from the mountain slopes of Kleinmond, Caledon Division, were kept in wet vermiculite under ordinary laboratory temperatures from the end of April. Germination was very slow and at the end of July of that year only 4 per cent of the seeds had germinated. The experiment was then stopped on account of loss of most of the seeds through fungal attack. The undifferentiated condition of the embryo in the mature seed probably requires this long germination period.

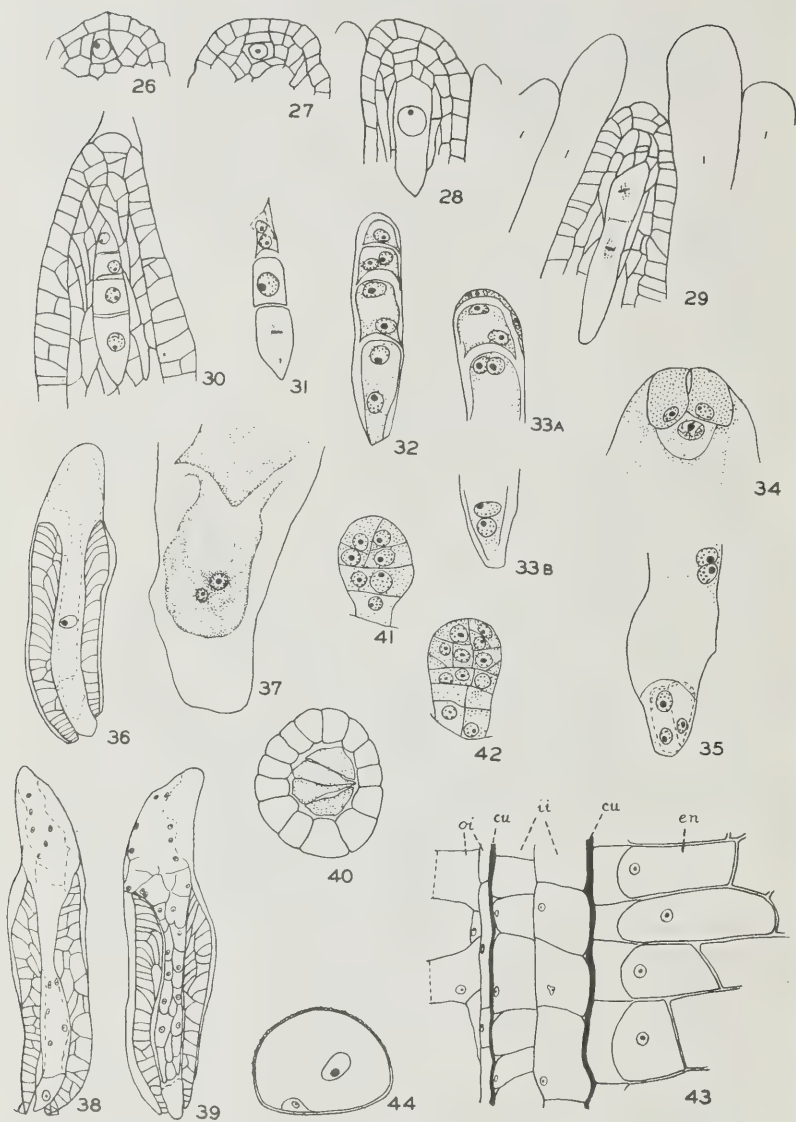
WACHENDORFIA PANICULATA BURM.

Before Dellert's paper on the embryology of *Wachendorfia paniculata* came to my notice, I sectioned and stained preparations to study the embryological development of this plant. As this series is complete showing stages not seen by Dellert, some supplementary information about the embryology of this plant is given. The present investigation confirms Dellert's observations, including some points about which she was not quite sure, e.g. that the pollen-grains are formed successively, that the parietal cell-complex she observed in the young ovule is formed from one primary parietal cell which is cut off from a subepidermal archesporial cell (Figs. 26—28), that the embryo-sac development is of the normal type, and the development of the endosperm helobial.

FIGS. 26—44, *Wachendorfia paniculata*, development of ovule and seed. FIG. 26, subepidermal archesporial cell; 27, megaspore-mother-cell and parietal cell; 28, enlarging megaspore-mother-cell and parietal cell-complex; 29, second metaphase of reduction division; nucellar epidermis two-layered at tip; 30, 31, rows of four megaspores; in 31 the upper megaspore lies upon the second one; 32, four megaspores, three of them with two nuclei; chalazal megaspore is functional; 33A & B, young embryo-sac with four nuclei: 33A, two nuclei in upper half, with disintegrating megaspore above them; 33B, lower half; 34, egg-apparatus; 35, lower half of mature embryo-sac with antipodal cells and polar nuclei; 36, embryo-sac after fertilization with a large upper and a small basal endosperm cell; 37, basal endosperm cell; 38, embryo-sac with a number of free endosperm nuclei in the upper chamber; 39, stage in cytokinesis, with cells in the lower half of the upper endosperm chamber and free nuclei in the top half; 40, chalazal haustorium in cross-section; 41, young embryo consisting of about twelve cells; 42, slightly older embryo with suspensor consisting of two cell rows; 43, section through seed-coat and outer layer of endosperm of nearly ripe seed; 44, young pollen-grain with generative and vegetative cell. FIGS. 26—35, 37, 41, 42, $\times 350$; 36, 38, 39, $\times 80$; 40, $\times 200$; 43, $\times 250$; 44, $\times 400$; *cu*, cuticular layer; *en*, endosperm; *ii*, inner integument; *oi*, outer integument.

The following describes some of the embryological characteristics not observed by Dellert:

Pollen.—In the young pollen-grains the generative cell which is small and more or less pear-shaped, is formed against the distal wall nearer one corner (Fig. 44).



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Ovule.—The ovule is hemitropous and sessile. Four megaspores are formed in a row (Figs. 30, 31). The upper one, however, often lies slightly out of line in an oblique position (Fig. 31) on account of the second reduction division spindle in the upper interphase cell being oblique (Fig. 29). This is probably the reason why Dellert could not find more than three megaspores in her material. Often more than one megaspore starts developing, e.g. in Fig. 32, where three of the megaspores have two nuclei. The chalazal one usually gets the upper hand and the other megaspores degenerate very slowly, so that in some ovules where the embryo-sac is in the four-nucleate stage, remains of them are still visible (Figs. 33A and B).

Dellert's suggestion that the development of the endosperm is most probably helobial, is correct (Figs. 36, 37). In the upper endosperm chamber which is formed after the first division of the primary endosperm nucleus, a large number of free nuclei are formed (Fig. 38). Cytokinesis starts from the base upwards, so that a few cases were seen with the lower half of the embryo-sac already in a cellular state, but the wider upper part still nuclear (Fig. 39).

The primary basal endosperm cell divides to form four large cells with hypertrophied nuclei and much cytoplasm. In a transverse section through the ovule these cells lie in a row (Fig. 40). They form a chalazal haustorium which gradually absorbs the nucellar cells that separate them from the vascular bundle in the chalaza, and later only some crushed cell-walls lie between them and the vascular bundle. The anti-podal cells degenerate early, and no postament is formed.

Dellert has suggested that the endosperm cells above the four cells of the chalazal haustorium, which in one stage look different from those near the micropyle, probably also arise from the primary basal cell. This, however, has not been confirmed. Although these endosperm cells at first look different from the upper ones, they also seem to originate from the free nuclei of the upper endosperm chamber (cf. Figs. 38, 39). The primary basal endosperm cell forms only the four cells of the chalazal haustorium.

The embryo-sac widens considerably as the endosperm develops, and later becomes reniform or sometimes oval in longitudinal section, with the embryo and micropyle at the lower end and the hilum in the middle of one side, the concave side when reniform. The chalazal haustorium later is pulled away from the chalazal region and lies in a space which is still to be seen in some of the mature seeds. Later the haustorial cells are absorbed together with some of the neighbouring endosperm cells.

The endosperm of the mature seed does not show the two regions seen earlier. Like *Dilatris* it is, however, differentiated into a peripheral

1-celled layer with thick outer walls which give a cellulose reaction, and an inner mass of cells which contain starch, protein and oil as reserves. The outer layer of endosperm contains protein but hardly any starch.

The earliest stages of the division of the zygote were not found. A later stage shows a young undifferentiated embryo with a short suspensor which consists of one, and a little later two rows of cells (Figs. 41, 42). In the ripe seed the embryo is differentiated and shows a slender terminal cotyledon and a shoot growing-point about half-way down its length. The suspensor remains short, stout and straight. In these points the embryo therefore differs considerably from that of *Dilatris*.

The seed is covered with brown bristly hairs or papillae which are formed by the cells of the outer integument growing out to form long tubular projections. The inner layer of this integument is very thin and the cells can hardly be recognized. The inner integument still consists of two cell-layers which have turned brown. Only two cuticular layers are present: between the two integuments and inside the inner one. The outer cuticle has disappeared on account of the external papillose protrusions from the outer epidermis. The nucellus has disappeared completely.

One, two, or all three seeds of the ovary may ripen, most frequently two.

DISCUSSION.

Relationship between Dilatris and Wachendorfia.—Tables II and III summarize the embryological characteristics of those genera of the tribes

TABLE II. THE MOST IMPORTANT EMBRYOLOGICAL CHARACTERISTICS OF GENERA OF THE TRIBES HAEMODOREAE, CONOSTYLEAE AND HYPOXIDEAE.

	Dila- tris.	Wachen- dorfia.	Xiphi- dium.	Anigos- anthus.	Hy- poxis.	Curcu- ligo.	For- besia. ¹	Ianthe. ²	Pauri- dia.
Periplasmodium in pol- len sacs	+	+	+	+	+	+	+ ³	+ ³	+ ³
Development of pollen	succ	succ	succ	succ	succ	succ	prob ³ succ	prob ³ succ	prob ³ succ
Integuments	2	2	2	2	2	2	2	2	2
Nucellus crassinucellate or tenuinucellate . .	cras	cras	cras	cras	ten	ten	ten	ten	ten
Parietal cell	+	+	+	+	0	0	0	0	0
Megaspores, number and arrangement . .	4, lin	4, lin		4, lin	4, T or lin	4, T	3	4, T	4, T
Embryo-sac develop- ment	NT	NT	NT	NT	NT	NT	Allium He	NT He,	NT
Endosperm development	He	He			He			Nu Nu	Nu
Chalazal haustorium . .	+	+					+	+ & O	0

¹ Now Empodium Salisb.

² Now Spiloxene Salisb.

³ de Vos, unpublished.

Haemodoreae, Conostylideae and Hypoxideae, where these characteristics have been worked out. In Table II such embryological characteristics are tabulated which have often been considered of importance to show relationships (cf., e.g. Schnarf and Wunderlich, 1939, and Cave, 1953), and in Table III some other points of similarity and difference are tabulated which, taken together with the points in Table II, also indicate relationships (Maheshwari, 1950, p. 357).

From these tables it is clear that the embryological characteristics of *Dilatriss* and *Wachendorfia* are remarkably similar, except for the shape of the ovule and the structure of the embryo and seed-coat (not tabulated). Embryological evidence therefore supports the placing of these two genera in one family, as systematists have always done in the past, or even one tribe, as Bentham and Hooker and Hutchinson have done—although the ovary is superior in *Wachendorfia* and inferior in *Dilatriss*.

TABLE III. SOME OTHER EMBRYOLOGICAL CHARACTERISTICS OF GENERA OF THE TRIBES HAEMODOREAE, CONOSTYLEAE AND HYPOXIDEAE.

	Dila- tris.	Wachen- dorfia	Xiphi- dium.	Anigos- anthus.	Hy- poxis.	Curcu- ligo.	For- besia.	Ianthe.	Pauri- dia.
Position of generative cell in pollen (distal or proximal) ..	dist	dist							
Funicle	0	0	short	short			+	+	+
Ovule	orth	hemi	orth	orth	ana	ana	hemi	ana	hemi
Micropyle directed downwards	+	+	±	±	—	—	—	—	—
Integuments largely 2- layered	+	+		+			more	+	+
Inner integument wider at tip	+	+					+	±	±
Integuments which form micropyle	inner	inner		both			both	both	both
Parietal cell forms several layers ..	+	+		±	0	0	0	0	0
Nucellar epidermis 2 or more-layered at tip..	+	+		—	±		+	+	—
Nucellar epidermal cells palisade-like towards base	+	±					—	—	—
Antipodals large, at same level	+	+		+			—	—	—
Antipodals degenerate	late	early		at fert	early		± late	late or early	late
Number of cells in chalazal haustorium	4	4			1		1	1 or 0	0
Embryo small in ripe seed	+	—		+	+		+	+	+
Suspensor	bent	2 cell rows		short	short?		short	short	short
Authority	present investi- gation	Dellert 1933 & present investi- gation	Stenar 1938	Stenar 1927	Stenar 1925		de Vos 1948, 1949		

TABLE IV. SOME MORPHOLOGICAL CHARACTERISTICS OF GENERA OF THE TRIBES HAEMODOREAE, CONOSTYLEAE AND HYPOXIDEAE.

	Dilatris.	Wachendorfia.	Xiphidium.	Anigosanthus.	Hypoxis.	Curculigo.	Forbesia.	Ianthe.	Pauridia.
Stamens	3	3	3	6	6	6	6	6	3
Ovary	inf	sup	sup	inf	inf	inf	inf	inf	inf
Ovules per chamber ..	1	1	∞	∞	∞	6—8	∞	∞	∞
Placenta	large	large	large	large	small	small	small	small	small

Of the two genera *Dilatris* shows more advanced characteristics, both morphological and embryological, viz., the inferior ovary, the very large placenta to which the ovule is attached by an extra strip of parenchymatous tissue, the flattened watchglass-shaped seed, the very pronounced palisade-like cells of the nucellar epidermis, and perhaps also the undifferentiated embryo.

Relationship between Dilatris, and Wachendorfia, Xiphidium and Anigosanthus.—Dellert has shown on embryological grounds that *Wachendorfia* stands close to *Anigosanthus*, and Stenar (1938) similarly shows that *Xiphidium* and *Anigosanthus* are closely related. In Tables II and III their results are summarized. Although the embryological development of *Xiphidium* and *Anigosanthus* has not been worked out fully, there seems to be enough evidence to agree with their views and, as shown above, to add *Dilatris* to this group of related genera.

The anatomical investigations of Schulze (1893) also showed the two Cape genera, *Wachendorfia* and *Dilatris*, to be related to the American genera *Xiphidium* and *Schiekia*.

The most important differences in the ovary and anthers known at present in the four above-mentioned genera are not embryological but morphological: (1) difference in the number of ovules per chamber. This need not be considered a character of great taxonomic importance and may only indicate which of the genera are more advanced in this respect. (2) Difference in the number of stamens. It has become evident that this also need not be considered of very great taxonomic importance in certain groups, as similar reductions have been found to occur within several other homogeneous groups of the Liliiflorae, e.g. in the Hypoxideae where *Pauridia* with three stamens is in all other respects (general morphology, anatomy, embryology, seed development and pollen morphology, cf. Schulze 1893, Brackett 1923, de Vos 1949, Erdtman 1952) similar to certain Cape *Spiloxene* (*Ianthe*) species. (3) Difference in the pollen morphology. *Wachendorfia*, *Xiphidium* and *Dilatris* have monosulcate grains with the sulcus sometimes operculate, and *Anigosanthus* has

"isopolar(?) to subisopolar 2-aperturate" grains (Erdtman 1952, pp. 46, 198). Erdtman does not state whether he considers these characters to be related and only refers to Hutchinson who places the Conostyleae (to which *Anigosanthus* belongs) in the Haemodoraceae "as a very distinct tribe".

The similarity in the embryological characteristics of these four genera is such that they must be closely related and it warrants their being placed in one family at least. This is therefore against Engler and Prantl's system (1930) in which *Wachendorfia*, *Dilatris* and *Xiphidium* are placed in the Haemodoraceae and *Anigosanthus* in the Amaryllidaceae-Hypoxidoideae, but supports workers like Bentham and Hooker and Hutchinson, who place all four genera in one family.

Unfortunately, these four genera are the only members of the tribes Conostyleae and Haemodoreae that have been examined embryologically. It is quite probable that some of the other genera of these two tribes will show somewhat similar embryological characteristics. In this respect it is interesting to note that *Conostylis* also has hemianatropous ovules according to Schnizlein (1860), and that *Haemodorum* has two hemianatropous ovules on thick placentae in each chamber, with a small embryo at a distance from the hilum (B. & H. III, p. 673). On the other hand, there is *Lanaria* which has been placed by Bentham and Hooker and by Hutchinson in the (Eu)haemodoreae and by Pax and Hoffmann in the Conostylideae. Preliminary investigations show that the embryology of this monotypic genus differs from that of the four genera discussed above and also from the tribe Hypoxideae. It has, for example, no periplasmodium and the pollen-grains develop simultaneously. More embryological investigations in these tribes are therefore necessary.

Relationship of the four genera with the Hypoxideae.—Dellert has also found embryological evidence to suggest that *Wachendorfia*, *Anigosanthus*, and the members of the tribe Hypoxideae can belong to one family. Stenar (1938) agrees and adds *Xiphidium* to the group, as mentioned above.

From Tables II and III some important points of similarity are evident between the Hypoxideae and the four genera of the Conostyleae-Haemodoreae group: (1) the presence of a periplasmodium which, of all the Liliiflorae, has been found in these genera only; (2) successive cell-wall formation in the p.m. cells; (3) the presence of two integuments (which is of such common occurrence in the Liliiflorae that not much importance can here be attached to it); (4) normal type of embryo-sac development; (5) helobial type of endosperm development, except for a few South African Hypoxideae. Dellert also mentions the antipodals

which degenerate early. But in *Dilatris* they degenerate late and therefore no similarity in this characteristic exists.

On the other hand some rather important differences between the embryological characteristics of the Hypoxideae and the Conostyleae-Haemodoreae group are evident, the most important being the absence of a parietal cell in the ovules of the Hypoxideae, the smaller size of the antipodals, and the smaller placentae. The pollen morphology also differs (Erdtman, 1952). Dellert discusses the first two points and concludes that they are not of such importance as are the points of similarity, and Schnarf (1931, p. 14) states that the nature of the antipodals has only a limited systematic value.

Pax and Hoffman (1930) place the four tribes, Alstroemerieae, Hypoxideae, Conanthereae and Conostylideae, in the subfamily Hypoxidoideae of the Amaryllidaceae. But the problem whether this subfamily is homogeneous, still needs further investigation. Comparative embryological and pollen investigations indicate a possible polyphyletic origin: the development of the pollen and ovule in the Alstroemerieae differs considerably from that of both the Hypoxideae and Conostylideae (Stenar, 1925, 1938 pp. 227-278). In anatomy the Alstroemerieae also differs from the Hypoxideae (Schulze, 1893, p. 382; Schnarf, 1892, p. 325). The embryology of the Conanthereae has not been worked out satisfactorily, the only genera investigated so far being *Odontostomum* (Cave, 1952) and *Cyanella* (de Vos, 1950). These investigations indicate that their development follows another pattern (or patterns). Further investigation is therefore still needed. The anatomy (Schulze, 1893) and the pollen morphology of the Conanthereae also differ: Erdtman (1952, p. 46) has stated that the pollen morphology of the Conanthereae supports the establishment of a separate family, the Tecophilaeaceae.

The embryological investigations so far done indicate that the three genera, *Wachendorfia*, *Dilatris* and *Xiphidium*, of the Haemodoreae and the one genus, *Anigosanthus*, of the Conostyleae stand closer to the Hypoxideae than to either the Alstroemerieae or the Conanthereae. According to the comparative embryological evidence available, the four genera and the Hypoxideae can possibly be included in one family.

OPSOMMING.

'n Oorsig word gegee van die afgrensing van die Haemodoraceae deur verskillende taksonome, asook van die literatuur oor die embriologiese ontwikkeling by genera van die familie. In sekere gevalle het hierdie kennis bygedra tot 'n beter begrip van die verwantskappe van die betrokke genera.

Embriologiese ondersoek by *Dilatris pillansii* toon dat die tapetum van die helmknoppe 'n periplasmodium vorm wat tussen die jong stuif-

meelkorrels indring, en dat die selwande van die jong stuifmeelkorrels suksessief neergelê word na die reduksiedeling. Die generatiewe sel van die stuifmeelkorrels word in een hoek teenaan die distale wand gevorm.

In die vrugbeginsel is daar in elke hok een sittende, ortotrope, kras-sinusellate saadknop met twee integumente aan 'n groot plasenta. Uit die subepidermale archespoorsel ontstaan 'n makrospoormoedersel en 'n dek-sel. L.g. deel om 'n klein dek-selle-kompleks te vorm bo die makrospoormoedersel en die makrospore. Die epidermis van die nucellus aan die top deel ook periklinaal. Na die reduksiedeling van die makrospoormoedersel word vier makrospore in 'n ry gevorm, en die chalazale een is funksionierend.

Die kiemsakontwikkeling is volgens die normale tipe. Die antipodeselle is groot en lank en degenereer eers na die bevrugting. Die endospermontwikkeling is volgens die helobiale tipe. 'n Klein basale sel word afgesny en daaruit ontstaan vier selle wat hipertrofie ondergaan en 'n chalazale haustorium vorm. In die boonste endospermcel ontwikkel die egte endospermweefsel, eers deur die vorming van 'n groot aantal vrye kerne wat later met selwande omgewe word.

Die embrio ontwikkel volgens òf die Asterad- òf die Crucifer-tipe. In die ryp saad is dit ongedifferensieer en die kort suspensor is gebuig. Die embrio lê in 'n klein uitstulping of sakkie van die kiemsak wat platgedruk is teen die endosperm. Die saadhuud is hoofsaaklik uit die buitenste opperhuud van die buitenste integument opgebou.

Meer informasie word gegee oor die embriologie van *Wachendorfia paniculata* wat alreeds voorheen ondersoek is (Dellert, 1933). Die embriologiese ontwikkeling van hierdie plant is in ooreenstemming met die van *Dilatriss*, behalwe dat die saadknop hemitroop is, en die kiem in die ryp saad gedifferensieer is, met 'n reguit suspensor wat uit twee rye selle bestaan; die bou van die saadhuud verskil ook effens.

Hierdie embriologiese studie toon dat (a) *Dilatriss* en *Wachendorfia* baie na verwant is, (b) hierdie twee genera ook na verwant is aan *Xiphidium* en *Anigosanthus*, en (c) dat die vier genera nader verwant is aan die tribus Hypoxideae as aan enige ander groep waarvan die embriologie uitgewerk is. Die plasing van *Dilatriss* en *Wachendorfia* in een tribus en van die vier genera in een familie, soos deur Bentham en Hooker en Hutchinson gedoen, word dus deur die vergelykende embriologie bevestig. Volgens die embriologie te oordele sou die vier genera en die Hypoxideae moontlik in een familie geplaas kan word.

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